OAK RIDGE NATIONAL LABORATORY
OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION
UNION CARBIDE
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OAK RIDGE, TENNESSEE 37830

February 14, 1975

Dr. John Kreisher
Associate Scientific Director
Council for Tobacco Research-USA, Inc.
110 East 59th Street
New York, New York 10022

Dear John:

I have attached my estimate of the accomplishments expected within the framework of existing third year funding and additional studies I believe worthy of supplementary funding as of 6-1-75.

Please let me know which, if any, of these additional studies should be included in a formal proposal for supplementary work/funding. I will prepare the formal request as soon as I hear from you.

You will note from Jim's Interim Progress Report that significant progress has been made. Our only complication has come in the major modifications and repair required of the LACS II and P&I SEM I.

You will be pleased to hear that considerable progress has also been made in preparing open literature publications. Considering deadlines, they will not appear in the literature before our more detailed Progress Report preparatory to third year funding is forwarded to you.

I look forward to hearing from you.

Sincerely,

M. R. Guerin

MRG
Attachment

APPENDIX I

PROJECTED CORE ACCOMPLISHMENTS*
(Without Supplementary Funding)

September 1975, 3 months into year 3

1. LACS II and P&I systems operational--at least well enough to test chemically.
2. Tests applied and results available as follows: mice present, ~10% smoke, 30/30 smoke/air cycle.

Measurement[1]	Purpose	Walton	P&I	LACS II
TC Monitor[2]	gp uniformity, age, purge	5 positions	7 positions	7 positions
Optical Monitor[2]	pm uniformity, age, purge	5 positions	7 positions	7 positions
GP Profile[3]	gp composition	1 position	1 position	1 position
TPM Profile[3]	pm composition	1 position	1 position	1 position
CO/CO_2	gp concentration, uptake/buildup	1 position	1 position	1 position
H_2O	humidity	1 position	1 position	1 position
Nicotine	pm concentration, nicotine uptake	1 position	1 position	1 position
Neophytadiene	pm concentration	1 position	1 position	1 position
Cost/Animal	cost/benefit	--	--	--
Mechanical	reliability, service requirements	--	--	--

(1) Relative to standard analytical smoke and nonrestrictive smoke.
(2) Continuous Measurement.
(3) Early and late (5 sec, 25 sec) in stand period.

December 1975, 6 months into year 3

1. Above, plus
2. Final report including following measurements: (a) DCBP or DTC dosimetry, (b) particle size growth and particle concentration.

June 1976, 12 months into year 3

1. Above, plus
2. Identification of constituents extracted preferentially from gas phase and particulate phase (determine whether change in composition is significant).
3. Particle size growth and concentration as functions of puff parameters, mode (normal, reverse; free, restricted) of puffing.
4. Complete comparative scheme, criteria, measurements.

APPENDIX II

"SUPPLEMENTARY" STUDIES

1. Special Measurements (BaP, HCN).

   a. BaP (Determination of trace constituent known to be carcinogenic). Method based on addition of carbon-14-labelled BaP to cigarette will be developed and applied to determine distribution of BaP in chamber(s). Determine whether measurements of nicotine, DCBP, DTC-C^{14}, and neophytadiene allow prediction of BaP concentration in unlabelled smoke and determine uptake by animals.

   b. HCN (Determination of known toxic and ciliatoxic constituent capable of reaction). Method based on standard colorimetric procedure or on nitrogen-selective gas chromatography will be developed and applied to determine concentration and distribution in chamber(s). Determine predictability of concentration from analytical measurements, loss to reaction if any, and uptake by animals.

2. Dosimetry by Depletion.

   It has been demonstrated (work for NCI, to be published) that the decrease in concentration of any constituent during the smoke stand period is a measure of the quantity of that constituent accepted by the animals, i.e., the dose of that constituent. Chamber depletion analysis thus allows determining the doses of actual smoke constituents and, being a non-destructive measurement, allows studying dose throughout a chronic exposure experiment.

   The proposed work:

   Using the mouse strain used in the Microbiological Associates chronic study, the depletions of nicotine, carbon monoxide, acetaldehyde, acrolein, isoprene, hydrogen cyanide (if 1-b is approved), catechol, glycerol, palmitic acid, phenol, and neophytadiene will be determined. Exposure conditions, including the test cigarette, will be the same as employed in the MICRO study.

   Above data will be studied to determine whether depletions of nicotine and carbon monoxide can be used to predict doses of other constituents and whether a constituent other than nicotine is a preferred particulate matter indicator for comparing exposure systems. Data will define MICRO exposure.

3. Multiple Inhalation Dosing

   Studies of the contribution of individual smoke constituents or smoke fractions to observed biological effects resulting from inhalation exposure require that techniques and instrumentation be available to allow multiple dosing. Studies of co-carcinogenic activity and individual susceptability also require or at least benefit from the availability of such instrumentation.

   Two approaches are possible. A pure compound, e.g., BaP, can be added to the cigarette to increase the concentration of that constituent in the smoke. Alternately, the compound or mixture can be metered into the chamber with the diluting air. The preferred approach depends on the experimental objectives--gases are best metered with the diluting air while some particulate components are best added to the cigarette.

The proposed work:

A carbon-14 distribution system of the type described by Philip Morris will be constructed and a carbon-14 sensitive gas chromatographic system will be purchased to allow studies of the fate of smoke constituents (selected for interest in co-factor exposure) added to the cigarettes. Constituents found transferred to the mainstream smoke without decomposition will be documented as applicable to this mode of co-exposure. Sufficient quantities of the constituent to be added will be synthesized or purchased and purified to prepare spiked cigarettes for inhalation testing.

The Walton-Horizontal system will be studied for the practicality of four co-factor dosing approaches: (a) pre-admission of freshly generated gas phase of smoke, (b) addition of gases and low molecular weight organics to fresh air supply, (c) pre-admission of gases and low molecular weight organics, and (d) pre-admission of particulates, smoke and non-smoke related, using an aerosol generator. Studies will include the development of suitable methods and hardware to produce the co-factor dose and chemically determining the concentrations and stability of the co-factor during the exposure period.

Co-factors to be studied using either cigarette addition and/or independent introduction will include carbon monoxide (stable gas), nitric oxide (reactive gas), acetaldehyde (low molecular weight organic), BaP (particulate carcinogen), smoke gas phase, classic tumor promoters, and an inorganic/organic aerosol yet to be selected.

4. Special Services for Microbiological Associates.

A "complete" chemical characterization of the smoke produced from the cigarette used for biological studies at MICRO would provide the following advantages: (a) direct comparability with NCI studies of approximately 120 cigarettes, and (b) "complete" definition of the exposure medium.

The proposed work:

Using both standard analytical smoke generation and standard but non-restricted analytical smoke generation, the following constituents will be determined in duplicate in the smoke of one cigarette type: TPM, water, nicotine alkaloids, tar, acetaldehyde, acrolein, isoprene, formaldehyde, oxides of nitrogen, hydrogen cyanide, carbon monoxide, carbon dioxide, whole smoke pH, catechol, phenol, free fatty acids, titrimetric acids, glycerol, and neophytadiene. Condensate (assumed produced by Meloy Labs under separate CTR contract) will be analyzed for indole, skatole, titrimetric acids, pH, BaA, BaP, phenol and cresols.

5. Subfractionation of Condensate by Chromatography--Pilot Study.

Future work for CTR-USA at this Laboratory will increasingly address quantitative fractionation of condensed smokes in support of biological studies by other CTR-USA contractors. A promising option to the sometimes criticized "Stedman-type" fractionation is the "SARA" chromatographic fractionation approach developed specifically for petroleum-related samples.

The proposed work:

The "SARA" chromatographic separation system will be constructed, applied to a petroleum-related sample to insure proper operation, and applied to condensed cigarette smoke. Aliquots of a given condensate will be subjected to the separation at least three times and the fractions obtained will be analyzed to determine quantitative and qualitative reproducibility. It will be known

whether the approach shows sufficient promise to warrent efforts to modify the systems for preparative scale operations.

APPENDIX II (page 3)

COST ESTIMATES

Supplementary Study	Man Year Ph.D	Man Year BS/MS	Man Year Tech.	Construction, Supplies, Equipment	Total Cost $/year	Completion Years
1. Special Measurements (BaP, HCN)	0.05	0.50	--	0.8 K	28.3 K	1.0
2. Dosimetry/Depletion	0.05	--	0.50	6.5 K	24.0 K	1.0
3. Multiple Dosing	1.00	1.00	--	25 K	125 K	2.0
4. Special Services, MICRO	0.05	--	0.50	0.5 K	18.0 K	1.0
5. SARA Fractionation	0.10	1.00	--	8.0 K	63 K	1.0
	1.25	2.50	1.00	40.8 K	258.3 K	

Cost Supplement	$258,300
Gas Chrom-Mass Spec	110,000
TOTAL COST	$368,300

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